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NOTE

Pathology

Feline-type cystic basal cell tumor filled with abundant melanin pigment-rich fluid in a dog

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ABSTRACT. A 2-year-old castrated male mongrel dog presented with a well-demarcated fluctuant dermal mass, located on the back of the neck. Grossly along with cystic structures filled with a black greasy fluid, when cut open. Microscopically, the mass was multi-lobulated. The lobules consisted of neoplastic basaloid cells and showed central degeneration, forming multiple central cystic structures filled with dark melanin-pigmented materials. Immunohistochemically, the neoplastic cells were strongly positive for CK14 and partially positive for CK19, but negative for CK7, CK8/18, CD34, S-100, Melan-A and α-SMA. Based on the findings, the present case was diagnosed as a feline-type basal cell tumor characterized by cystic structures filled with abundant black fluid.

KEY WORDS: basal cell tumor, dark fluid, dog, feline, melanin

Basal cell neoplasm is a basaloid epithelial neoplasm without epidermal or adnexal differentiation [2, 3]. Previously, this type of neoplasm was also known as basal cell epithelioma and mainly classified into 4 major subtypes, such as solid, cystic, ribbon, and medusoid, in dogs and cats. This type of basal cell tumor has a wide range of host specificity in animals such as cats, dogs, horses, and sheep [11]. However, the basal cell tumor subtypes, except for cystic type, have been reclassified as a trichoblastoma and subclassified mainly into 6 major subtypes such as ribbon, medusoid (medusa), solid, granular, trabecular, spindle types in dogs and cats due to newly developed individual keratin markers for immunohistochemistry [3, 4]. The only left basal cell tumor (cystic-type basal cell tumor which is not reclassified into trichoblastoma) is currently known to be common in cats, and rare in other species [4]. Consequently, all types of previous canine basal cell tumor categories were reclassified as canine trichoblastoma according to the most recently revised classification of basal cell tumors [3], suggesting feline type cystic basal cell tumor is the only remaining category in the current basal cell tumor classification. Despite this reclassification, we recently observed a single feline-type basal cell tumor in a dog, which is not well-matched with the current system of basal cell tumor and trichoblastoma classification. Here, we describe an interesting case of feline-type cystic basal cell tumor in a mongrel dog for the first time.

A 2-year-old castrated male mongrel dog was admitted to a local animal hospital with a dark dermal mass on the back of the neck (Fig. 1A). The dermal mass was well demarcated, fluctuant, and showed mild alopecia of the overlying skin. Due to the fluctuation of the mass, the mass was thought to contain abundant fluid. Despite repeated drainage, the mass continuously increased in size for 1 year. Finally, the dark mass with diameter of 7 × 5 cm was surgically excised and sent to the Department of Veterinary Pathology, Teaching Animal Hospital, College of Veterinary Medicine, Kyungpook National University for histopathological diagnosis. The resected mass was fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. Paraffin blocks were cut into 5 μm-thick sections and stained with hematoxylin and eosin (HE) for histopathological

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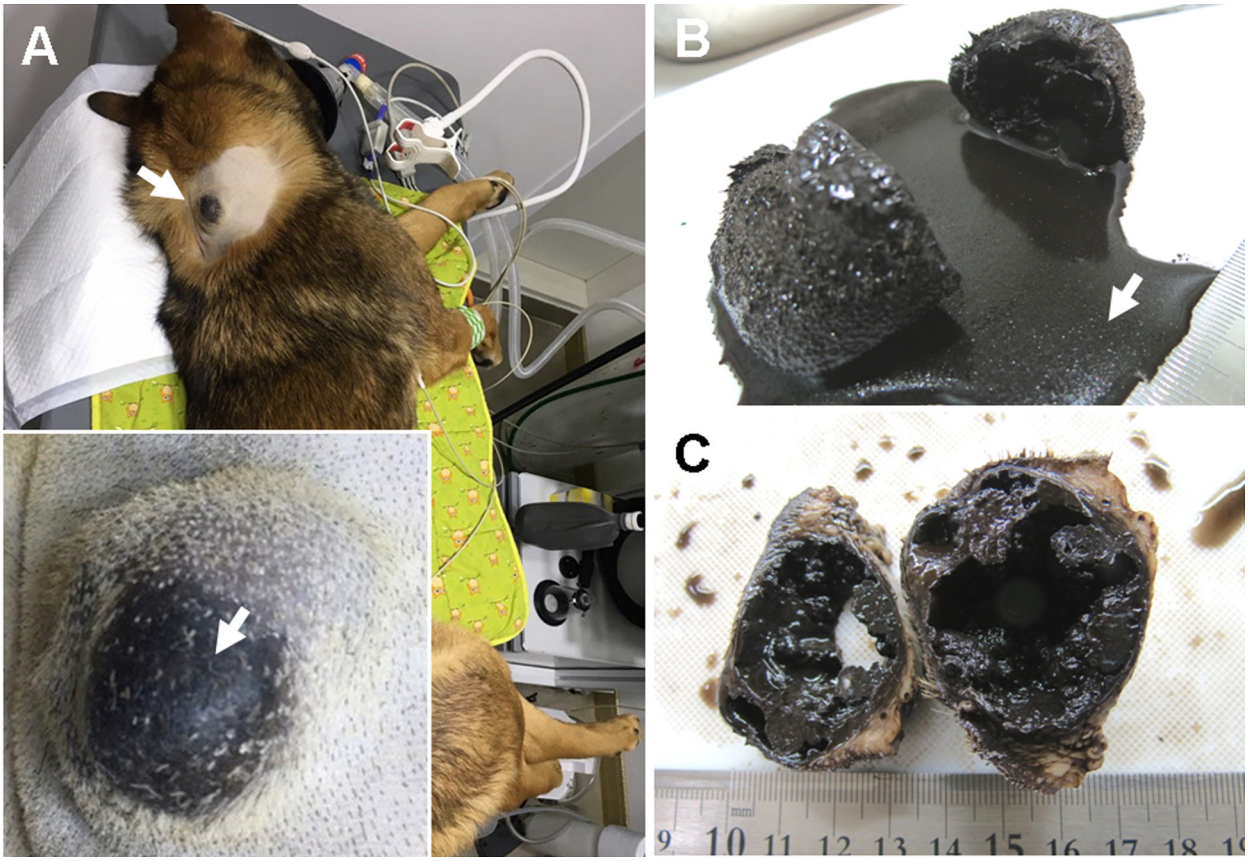

Fig. 1. Dermal mass on the back of the neck from a 2-year-old castrated male mongrel dog. A. A dark 7 × 5 cm dermal mass on the back of the neck (arrow). The mass was well demarcated, fluctuant, and showed mild alopecia (inset, arrow) on the overlying skin. B. Resected cutaneous mass filled with dark black greasy (arrow) fluid. C. Cut surface of the skin mass showing multiple cystic structures with amorphous dark black components.

examination. For a differential diagnosis, immunohistochemistry was performed. Serial sections were treated with 3% hydrogen peroxide (H_2O_2) in methanol at room temperature for 30 min and steamed for 30 min for antigen retrieval. The sections were then incubated with blocking solution (Life Technologies, Frederick, MD, U.S.A.) at room temperature (25°C) for 1 hr and treated with the primary mouse monoclonal antibodies including cytokeratin (CK) 7 (OV-TL 12/30, 1:200, Dako, Glostrup, Denmark), CK14 (LL002, 1:200, Serotec, Düsseldorf, Germany), CK8/18 (NCL-5D3, 1:200, Novocastra Laboratories, Newcastle, U.K.), CK19 (BA17, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), α-smooth muscle actin (α-SMA) (1A4, 1:500, Sigma, St. Louis, MO, U.S.A.), CD34 (B-6, 1:100, Santa Cruz Biotechnology, Dallas, TX, U.S.A.), Melan A (MLANA/788, 1:200, Novus, Littleton, CO, U.S.A.) and rabbit polyclonal antibody S-100 (1:5,000, Abcam, Cambridge, MA, U.S.A.) at 4°C overnight. After being washed three times in phosphate-buffered saline (PBS), the sections were incubated with a broad-spectrum secondary antibody (Life Technologies, Frederick, MD, U.S.A.) and horseradish peroxidase (HRP)-conjugated streptavidin (Life Technologies, Frederick, MD, U.S.A.) at room temperature for 10 min. Finally, the sections were visualized using a DAB peroxidase substrate kit (Vector Laboratories, Burlingame, CA, U.S.A.) and followed by counter staining using 10% hematoxylin for 3 min.

Grossly, the resected 7 × 5 cm cutaneous mass was very soft. When the mass was cut, it was filled with a dark black greasy fluid (Fig. 1B) and the cut surface showed multiple cystic structures with amorphous dark black components (Fig. 1C). Microscopically, the mass was multi-lobulated, divided by thin fibrous tissue. Many individual lobules of the neoplastic cells showed central degeneration and necrosis forming multiple central cysts in the lobules (Fig. 2A). These cystic structures contained homogeneous granular eosinophilic or blackish melanin-pigmented debris (Fig. 2A and 2B). Interestingly, the dark melanin-pigmented proteinaceous material showed numerous cholesterol clefts (Fig. 2B), which was well-matched with the gross finding of a greasy dark fluid (Fig. 1B). Numerous melanophages and melanin granules were also observed in the fibrous tissue between the neoplastic lobules (Fig. 2C). The neoplastic lobules were composed of basophilic basaloid cells characterized by round-to-ovoid nuclei, prominent nucleoli, scant cytoplasm, and oval-to-polyhedral shape (Fig. 2D). At the periphery of the neoplastic lobules, the basaloid cells showed a palisading arrangement attached to the basement membrane with occasional stromal-epithelial clefts (Fig. 2C inset and 2D). Mitotic figures were also frequently observed (4–5 mitotic figures/400X field). There was no adnexal or ductal differentiation. Melanocytes were observed frequently between the basophilic basaloid cells, with pigmentation of the neoplastic basaloid cells (Fig. 2D). Immunohistochemically, the neoplastic basaloid cells showed strongly positive for CK14

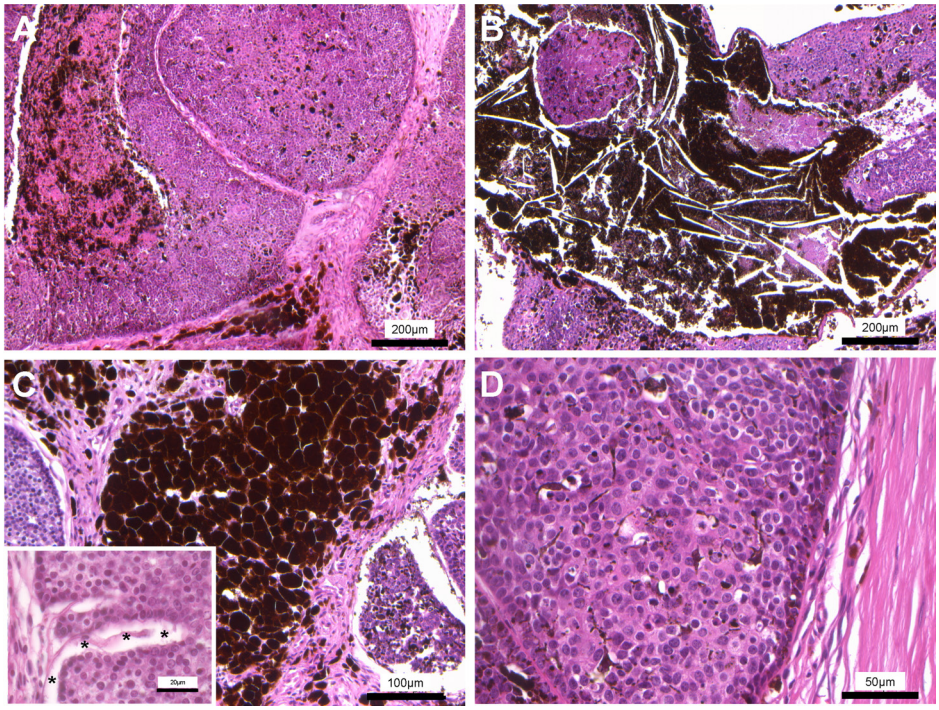

Fig. 2. Representative photomicrographs of microscopic findings. A. The mass was multi-lobulated with thin dividing fibrous tissue. Individual lobules of neoplastic cells showed central degeneration forming central cysts containing homogeneous granular eosinophilic and blackish melanin-pigmented debris. Hematoxylin & eosin (H&E) stain. Bar=200 μm. B. Numerous cholesterol clefts in melanin-pigmented proteinaceous materials. H&E stain. Bar=200 μm. C. Numerous melanophages and melanin granules in the interlobular fibrous tissues. H&E stain. Bar=100 μm. *Inset*: A palisading arrangement of neoplastic basaloid cells attached to the basement membrane with stromal-epithelial clefts (asterisks). H&E stain. Bar=20 μm. D. Neoplastic basaloid cells showing a palisading arrangement at the periphery of a neoplastic lobule. H&E stain. Bar=50 μm.

(Fig. 3A and 3B) and partially positive reactions for CK19 (Fig. 3C). However, the neoplastic cells were completely negative for CK8/18 (Fig. 3D), CK7 (Fig. 3E), CD34 (Fig. 3F), S-100 (Fig. 3G) and Melan-A (Fig. 3H). There was no α- SMA positive myoepithelial lining around the neoplastic lobules and only blood vessels exhibited a positive reaction for α- SMA (Fig. 3I).

Basal cell tumors are a common cutaneous epithelial non-adnexal neoplasm in the veterinary field, especially in cats [12]. They usually present as a round, solid, firm to cystic fluid-filled dermal mass [1]. Of all tumors, 25% are described as black or gray masses [12]. The most common locations of occurrence are the head and neck [1]. Feline basal cell tumors have several microscopic variations but the cystic pattern is the most frequently seen variant among several less common variations [1]. In the cystic pattern, the cysts generally contain homogeneous amorphous-to-granular eosinophilic proteinaceous fluid, as well as dark melanin pigmentation [1], which is well-matched with the histological findings in the present case. The present case can be misdiagnosed as one of many other epithelial tumors because of the morphological resemblance of basaloid tumors. For this reason, histological differential diagnoses for tumors of this type should include apocrine- and hair follicle-originating tumors, in particular trichoblastoma and apocrine ductal adenoma.

In dogs, what were previously referred to as "basal cell tumor" in the veterinary literature were reclassified as canine trichoblastoma because of immunohistochemical findings suggesting a hair germ cell origin [5]. Generally, canine trichoblastoma has six major histologic patterns, solid, medusoid, ribbon, trabecular, granular cell type, and spindle cell type, which were reclassified from the previous canine basal cell tumor category [3]. According to the current classification of canine basal cell tumors, however, the present case cannot be suitably categorized [3]. It seems to be more closely associated with feline type cystic basal cell tumors based on histological findings, such as central cystic degeneration, multi-lobular structure, and absence of adnexal differentiation. Thus, trichoblastoma was ruled out due to the histological characteristics of the present case according to the current classification of canine tumors.

Recently, feline basal cell tumors were also newly divided into two categories owing to the positive immunohistochemical reactions of cells around the lumen for CK7/8 (CAM5.2), indicating apocrine differentiation [3]. This led to the classification of tumors as either apocrine ductal adenoma with ductal differentiation or without ductal differentiation [3]. Basal cell neoplasms without ductal differentiation retain their basal cell tumor designation [3]. This suggests that basal cell tumors with ductal differentiation were reclassified as apocrine ductal adenomas, histologically characterized by ductal differentiation of the tumor cells, and have been completely separated from original basal cell tumor category in the cat. It is difficult to differentiate basal cell tumors from apocrine ductal adenoma in feline species because of their morphological similarity. Generally, feline basal cell

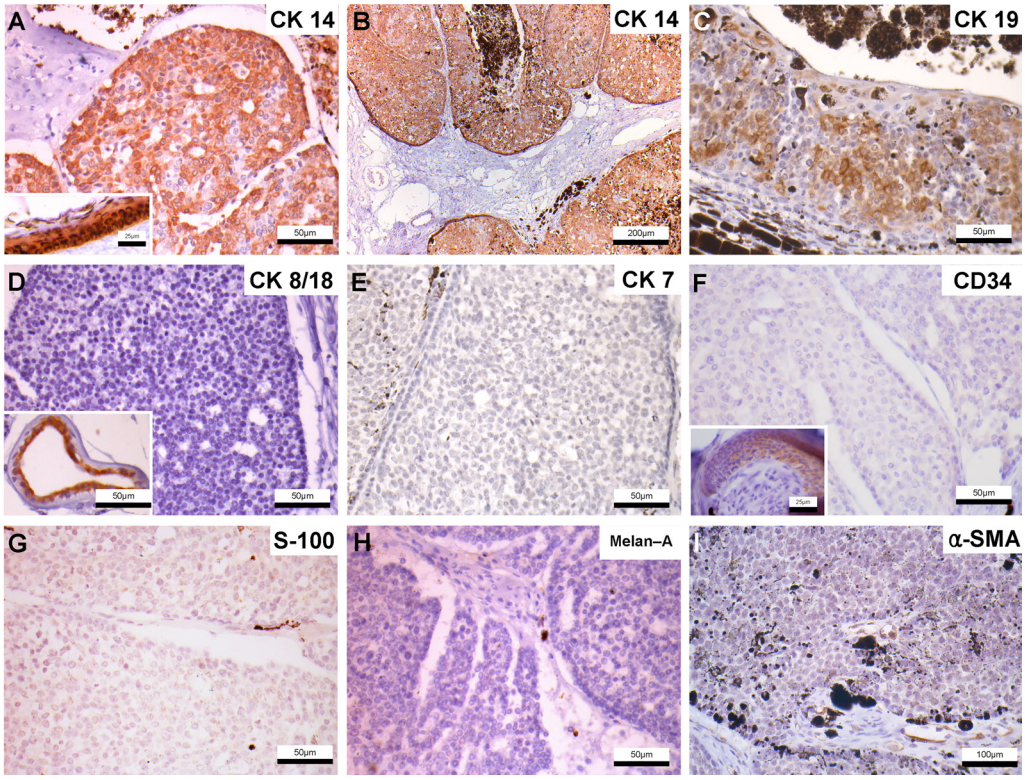

Fig. 3. Representative photomicrographs of immunohistochemistry. A. Strong positive reaction for CK14 in neoplastic basaloid cells. Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=50 μm. *Inset*: CK14 was also highly expressed in epidermal basal layer. Bar=25 μm. B. CK14 was highly expressed in over all lobules of neoplastic basaloid cells. Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=200 μm. C. Partially CK19 positive tumor cells. Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=50 μm. D. Negative reaction for CK8/18 in neoplastic cells Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=50 μm. *Inset*: CK8/18 was strongly expressed in normal apocrine gland. Bar=50 μm. E, F, G, H. Negative response of CK7, CD34, S100 and Melan-A in neoplastic basaloid cells. Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=50 μm. Fig. F *Inset*: Positive response of CD34 in hair follicular basal cells. Bar=50 μm. I. Absence of α-SMA positive myoepithelial lining around the neoplastic lobules except for positive reactions found only in blood vessels. Avidin-biotin-peroxidase method, hematoxylin counterstain. Bar=100 μm.

tumors show prominent nucleoli, horizontally developed mass, thick fibrous septa separating neoplastic lobules of tumor cells, formation of cystic structures filled with dark melanin-pigmented fluid, and the absence of ductal formations [1, 3, 5]. The present case demonstrated histological features matching the above detailed findings of feline basal cell tumors rather than feline apocrine ductal adenoma.

Immunohistochemically, the tumor cells of the present case returned negative findings for CK7, CK8/18, Melan-A, S-100, and α- SMA, suggesting that the tumor cells did not originate from melanocyte and apocrine epithelium [8, 15] due to the absence of myoepithelial cells around the neoplastic lobules [6]. However, interestingly, the tumor cell showed a strong positive reaction for CK14 and partially positive reaction for CK19, suggesting those tumor cells originated from basal cells of the epidermis [7, 9, 13] or hair follicular stem cells [10]. Since the tumor cell showed completely negative reactions for CD34, the possibility of a hair follicular stem cell origin was excluded [14]. Based on the above mentioned histological features and immunohistochemical results of the present case, it is strongly believed that the tumor cells originated from the basal cells of the epidermis. Therefore, apocrine ductal adenoma, as well as trichoblastoma, was ruled out in the present case.

Based on the gross, histopathological, and immunohistochemical findings, the present case was diagnosed as feline-type cystic basal cell tumor in a dog. To the authors' knowledge, there has not been any complete report of feline-type cystic basal cell tumor in dogs thus far. Since neoplasms previously classified as a basal cell tumor in the dog have been reclassified as a trichoblastoma [2, 3], the presence of this case may provide informative evidence suggesting that cystic type basal cell tumor should be considered for the diagnosis of melanin pigmented cutaneous tumors in dogs as well as cats in the future.

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